

Nanoscale Patterning Of Photosynthetic Light Harvesting Proteins

[N. Reynolds¹](#), [S. Janusz¹](#), [C.N. Hunter²](#), [G.J. Leggett¹](#)

¹ *The Department of Chemistry, The University of Sheffield, UK.* ² *Molecular Biology and Biotechnology, The University of Sheffield, UK.*

INTRODUCTION: Here we present the patterning of light harvesting 2 (LH2) complexes from the photosynthetic bacterium *Rhodospirillum rubrum*. LH2 complexes consist of circular arrays of bacteriochlorophyll and carotenoid molecules, held together by a cylindrical assembly of polypeptides. As many as 100 LH2 complexes are organized in the membrane to form an interconnected energy transfer network comprising thousands of bacteriochlorophyll molecules that absorb photons, channeling the excitation energy down an energy gradient towards the reaction centre (RC), leading to a charge separation that drives subsequent biosynthetic reactions in the cell. When removed from the photosynthetic membrane, LH2 complexes retain the ability to absorb light, and they emit the energy as fluorescence. This property has been utilized in order to gain insight into the biological functionality of the LH2 after immobilization on to nanoscale patterned surfaces.

METHODS: Alkanethiol SAMs on gold surfaces have been used in conjunction with photolithographic techniques to produce patterned assemblies of LH2. Selective exposure of alkanethiols to UV light (wavelength 244 nm) leads to their photo-oxidation to alkylsulfonates, which may be displaced by a second thiol in a solution-phase process. The adsorption of LH2 onto SAMs with a variety of functional groups has been measured by surface plasmon resonance (SPR) in order to determine which surfaces resist non-specific adsorption. In contrast to plasma proteins, which adsorb strongly to most surfaces, simple patterns consisting of hydrophilic and hydrophobic regions may be used effectively to pattern LH2. Covalent attachment to carboxylic acid groups using carbodiimide activation methods is an effective means of immobilizing LH2 at the surface. Fluorescence spectroscopy measurements of proteins immobilized by attachment to patterned SAMs were used to determine the biological activity of the complexes once immobilized to the surface. Nanoscale chemical patterns have been fabricated using scanning near-field photolithography (SNP), in which a scanning near-field optical microscope coupled to a UV laser is used to selectively expose regions of a SAM

RESULTS & DISCUSSION: The fluorescent spectroscopy results have confirmed that biological function is retained, leading to the observation of absorption spectra qualitatively identical to those of complexes in solution. Using SNP, lines of carboxylic acid functionalized thiols as small as 70nm have been fabricated in monolayers of perfluorinated thiols and used to form LH2 structures with a width of less than 100nm. Methodologies such as this, that are based on near-field photolithography offer great promise for the fabrication of functional, nanostructured assemblies of membrane proteins.

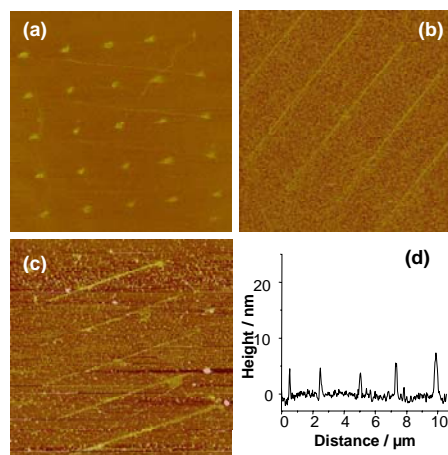


Table 1. : AFM images of nanoscale patterns fabricated by SNP¹. (a) 12 μm x 12 μm FFM image of an array of C₁₀COOH dots in a C₂F₅CF₃ monolayer. (b) 15 μm x 15 μm FFM image of C₁₀COOH lines in a C₂F₅CF₃ monolayer. (c) 15 μm x 15 μm tapping mode image of LH2 immobilised onto C₁₀COOH lines (z-range 0 – 40 nm). (d) cross-section across the nanolines in (c) with a mean FWHM of 98nm.

REFERENCES: 1. Reynolds *et. al.*, submitted, 2007